Purpose: It is widely accepted that the mechanical environment surrounding cartilage cells (chondrocytes) has a regulatory role on their metabolism. Very early osteoarthritis (OA) is associated with micro-cracks of the cartilage surface that are thought to alter the mechanical environment of chondrocytes, and hence their metabolism. In this study, we assessed the differences of cartilage deformation patterns between intact and cracked cartilage.
Methods: Articular cartilage split line patterns of New Zealand white Rabbits were identified using India-ink. Cracks were made at full thickness of cartilage at 90° to the cartilage surface and oriented perpendicular to the split lines. A controlled load of 2MPa was applied, and stress relaxation observed. Cartilage tissues were then fixed in the compressed state and prepared for histology. Samples were mounted either in plastic and stained with Toluidine blue or mounted in paraffin and stained with Picrosirius red for viewing under a polarized light microscope.
Results: The split line patterns of patella, femur, and tibia were found to be consistent. Histological analysis revealed that chondrocyte clusters in the radial zone show compression in the vertical direction and realignment of their vertical long axis to an oblique pattern pointing away from the center of the compression site. Under Linear Polarized Light (LPL), extensive collagen fiber reorientation is apparent as they deform in a combination of crimp and bending (Figure 1).

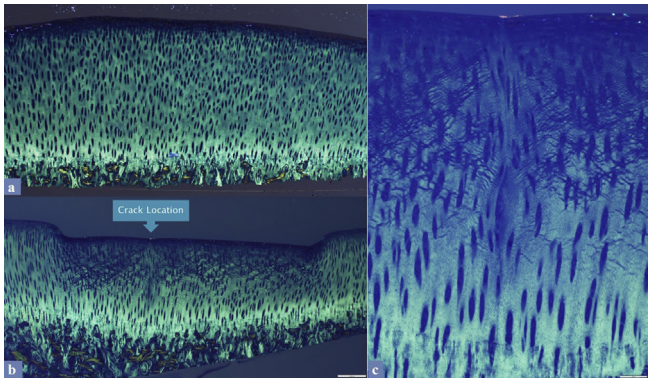


Figure 1, Rabbit tibia mounted in plastic, stained with toluidine blue and viewed under LPL. a, Uncompressed tibia. b, Compressed tibia showing the reoriented collagen fibers (fine blue lines). c, Crack location magnified. Under Circular Polarized Light (CPL), the pattern of collagen fiber reorientation that occurred in the cracked vs. the intact samples is different. There is an upside triangle-shaped zone with the crack representing the center. Collagen fibers at the side of the crack are less reoriented compared to those located farther away, indicating the effect of stress release at the crack edge. The sides of the triangle run at an angle similar to that of the oblique running fibers that are normally present in the radial zone of the cartilage, hence this pattern may emphasize the role of these oblique fibers in supporting compressive loads (Figure 2).

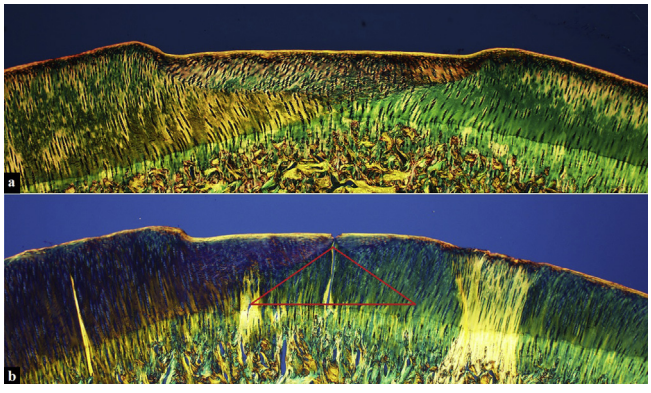


Figure 2, Rabbit tibia viewed under CPL. a, Intact compressed tibia cartilage. b, Cracked compressed tibia cartilage highlighting the upside triangle pattern.
Conclusions: The combination of crimp and bending of the collagen fibres during deformation may have a protective effect and reduce the amount of deformation transmitted to chondrocytes. The presence of a crack completely changed the deformation patterns in the collagen fibers. This is the first time where the micro-structural architecture and deformation of cracked cartilage under compressive loading has been quantified. The difference in collagen reorientation between intact and cracked samples may provide crucial insight into the load distribution in early OA cartilage that contains cracks, and may help identify how cracked cartilage degenerates. Structural changes in surface zone cartilage are among the earliest signs of very early OA and an understanding of these changes in mechanics and cell signaling may allow for altering the time course of OA by either mechanical or pharmacological intervention.

460
CARTILAGE IN THE CONTEXT OF HYPERGLYCEMIA AND DIABETES: FURTHER PATHOPHYSIOLOGICAL CLUES FOR DIABETES-RELATED OSTEOARTHRITIS

M.-C. Laiguillon †, A. Courties †‡, X. Houard †, M. Auclair †, A. Sautet §, J. Capeau †, B. Fève †, F. Berenbaum †‡, J. Sellam †‡. † *UMRS_938, Ctr. de Recherche St-Antoine, Sorbonne Université UPMC Univ Paris 06, Paris, France;* ‡ *Rheumatology Dept., Assistance Publique — Hôpitaux de Paris (AP-HP), Inflammation-Immunopathology-Biotherapy Dept. (DHU i2B), Paris, France;* § *Orthopedic surgery Dept., AP-HP, Sorbonne Université UPMC Univ Paris 06, Paris, France*

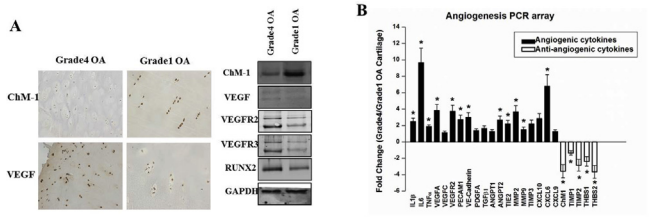
Purpose: Recent epidemiological studies have suggested an association between type 2 diabetes mellitus (DM) or chronic hyperglycemia and osteoarthritis (OA) but experimental evidences are lacking. We aimed i) to characterize ex vivo cartilage explants from type 2 DM OA patients versus non DM OA patients ii) to decipher in vitro the impact of high glucose environment on chondrocytes activation.
Methods: Ex vivo, the release of interleukin-6 (IL-6) and prostaglandin E2 (PGE2) was measured by ELISA/EIA in 24h-conditioned media of IL-1β-stimulated (5 ng/mL) OA cartilage from DM (n=5) and non-DM (n=5) patients. In vitro, primary cultures of murine chondrocytes were stimulated for 24 and 72h with/without IL-1β (5 ng/mL) in a normal (5.5 mM) or high (25 mM) glucose environment. Gene expression and release of pro-inflammatory mediators (IL-6, COX2/PGE2) were analyzed by RT-qPCR and ELISA/EIA. Glucose uptake was assessed with (14C)-2-deoxyglucose. Osmotic stress was assessed by mannitol addition experiments. Reactive oxygen species (ROS) and nitric oxide (NO) production were measured by fluorescent DCFDA assessment and by Griess reaction, respectively. To analyze the mechanism of IL1β-induced inflammation, cells were pretreated or treated with inhibitors of glucose transport (cytochalasin B, 1 μM), of the polyol pathway (epalrestat, 10 μM), mitochondrial oxidative stress (Mitotempo, 50 μM) or of NO-synthase (L-NAME, 5 mM).
Results: Human DM OA cartilage explants released more IL-6 (2.7-fold) and PGE2 (3-fold) than non-DM cartilages after IL-1β stimulation (p=0.047 and p=0.02, respectively). In cultured chondrocytes, after IL-1β stimulation, IL-6 and COX2 mRNA expressions and IL-6 and PGE2 releases were higher when cultured in high than normal glucose concentration (5.6- [IL-6 mRNA] and 3- [IL-6 protein], 8- [COX2 mRNA], 3.6-fold [PGE2]) (n=5, p=0.03). Glucose uptake was confirmed in normal condition but was also transiently increased with IL-1β at 72h (n=3). Mannitol experiments ruled out the hypothesis of an osmotic stress due to high glucose (n=4). High glucose significantly increased ROS and NO production induced by IL-1β as compared to normal glucose (2.1- and 1.9-fold, respectively; n=5, p=0.04). IL1β-induced IL-6 release was reduced by cytochalasin B, epalrestat or L-NAME treatment (-45%, -62% and -38%, respectively, n=5, p=0.04). A trend for a reduction of IL-6 production was also observed with Mitotempo (-40%, n=4, p=0.06).
Conclusions: OA cartilages from DM patients displayed an increased sensitivity to IL-1β-induced inflammation. Accordingly, high glucose enhanced IL-1β-induced inflammation in cultured chondrocytes by acting through oxidative stress and the polyol pathway. These results provide arguments for high glucose and diabetes participating to increased inflammation in OA.

461
RELATIVE PERIARTICULAR BONE MINERAL DENSITY ASSOCIATES WITH ARTICULAR CARTILAGE DAMAGE CROSS-SECTIONALLY AND LONGITUDINALLY

G.H. Lo †, E. Schneider ‡, J.B. Driban §, L. Price §, M. Zhang §, D. Harper §, C.B. Eaton ||, T.E. McAlindon §. † *Baylor Coll. of Med., Houston, TX, USA;*

‡ *Cleveland Clinic, Cleveland, OH, USA;* § *Tufts Med. Ctr., Boston, MA, USA;* ‖ *Mem. Hosp. of Rhode Island, Pawtucket, RI, USA*

Purpose: The anatomic and biomechanical properties of peri-articular bone play a primary role in the dispersion of loading forces across the joint. 30-50% of a load across a knee is absorbed by peri-articular bone compared to only 1-3% by articular cartilage. Attenuation of loading forces by peri-articular bone is critical in protecting articular cartilage from damage. However, thickening of subchondral bone and disruption of peri-articular trabecular architecture occur early in osteoarthritis (OA), and may even antedate cartilage damage. The purpose of this study is to evaluate whether relative periarticular bone mineral density is associated with MRI-assessed articular cartilage damage.
Methods: This is a study nested within an ancillary study to the Osteoarthritis Initiative (OAI). We selected a sample enriched with participants likely to have cartilage damage over time. Due to logistics of deploying the study within an ongoing observational study, the visit for the dual x-ray absorptiometry (DXA) measurements was the OAI 30 or 36 month visit and the visits for cartilage assessments were the OAI 24 and 48 month visits. DXAs of the knee generated two measurements, medial tibial or periarticular bone mineral density (medial paBMD) and medial:lateral periarticular bone mineral density (M:L paBMD) measures, using proprietary software. DH measured the cartilage damage index (CDI) in the medial tibiofemoral compartment 3T MRI acquired DESS sequences. The CDI is a parsimonious measure of cartilage damage in 9 informative regions in the medial tibiofemoral cartilage. Only evaluating the one knee per person, we created scatter plots and performed Pearson's correlations of the periarticular bone mineral density assessments and (1) the 24 month CDI assessments and (2) rate of change in CDI defined as the CDI difference between 24 and 48 months divided by the time elapsed between those visits.
Results: 130 participants were included in the study with a mean age of 64 $\pm$9.1 years, 53% were male, mean BMI was 30.1 $\pm$4.8 kg/m^2. At the OAI 24 month visit, 6 (5%) had a Kellgren Lawrence score of 0, 11 (9%) with a score of 1, 35 (26%) with a score of 2, and 78 (60%) with a score of 3 and 1 (1%) with a score of 4.



The relationships between relative periarticular bone mineral density and CDI were stronger than those evaluating absolute bone mineral density.
Conclusions: Higher relative periarticular BMD is associated with greater articular cartilage damage in the medial tibiofemoral cartilage damage cross sectionally and longitudinally. Because we were able to find this association, this study also additionally provides construct validity to the cartilage damage index as a parsimonious measurement of articular cartilage. This study highlights the close relationship between periarticular bone and articular cartilage.

462
CHONDROMODULIN-1 AMELIORATES OSTEOARTHRITIS PROGRESSION IN VITRO AND IN VIVO

X. Zhang, R. Crawford, Y. Xiao. *Inst. of Hlth.and BioMed. Innovation, Brisbane, Australia*

Purpose: Articular cartilage is an avascular tissue by nature, in which resident chondrocytes maintain a stable phenotype that is resistant to hypertrophy and angiogenesis throughout life. Hypoxia is known to stabilize hypoxia-inducible factor-α (mainly HIF-1α and HIF-2α) and initiate an angiogenic signalling cascade. However, normal cartilage maintains avascularity under hypoxic conditions; and the abrogation of avascularity in cartilage is related to several joint diseases such as osteoarthritis (OA), indicating the important role of anti-angiogenic factors in normal cartilage homeostasis.
Chondromodulin-1 (ChM-1) is an anti-angiogenic protein endogenously expressed in cartilage and has recently been shown to stabilize the chondrocyte phenotype during cartilage tissue repair *via* an unknown mechanism. These findings suggested that anti-angiogenic factors had a critical role in maintaining the physiological functions of chondrocytes and prevent hypertrophy. Our aims in the present study were to clarify the role of ChM-1 during chondrocyte maturation, OA development and therapy, in an animal model, as well as to explore the mechanistic pathways of ChM-1 with a focus of the regulation of the HIF pathways.
Methods: ChM-1 expression during chondrocyte maturation was investigated in chondrogenic culture conditions in cells derived from cartilage and bone marrow. Lentivirus vectors carrying ChM-1 complementary DNA (LV-ChM-1) and ChM-1 siRNA were constructed to respectively over-express and down-regulate ChM-1. Human OA cartilage samples were collected and subjected to gene profiling of angiogenic and anti-angiogenic cytokines using Human Angiogenesis PCR Arrays (Qiagen, Australia). Tumor necrosis factor alpha (TNFα) was applied to mimic the inflammatory environment in OA. Articular cartilage cells (ACCs) over-expressing or under-expressing ChM-1 were exposed to TNFα, and the chondrogenic and hypertrophic markers (*RUNX2, COL10, MMP13, ALP* and *VEGF*) were assayed by qRT-PCR and western blot. To evaluate the effect of ChM-1 in OA development, an OA model was created in rats by surgical sectioning of the meniscus. For the mechanistic study, intracellular protein levels, nuclear accumulation and transcriptional activity of HIF-2α with ChM overexpression were evaluated by western blot and Chromatin-Immunoprecipitation (ChIP) assay, respectively.
Results: Compared with healthy cartilage, the expression of angiogenic factors was significantly upregulated and anti-angiogenic factors were suppressed in OA cartilage (Figure 1A). ChM-1 expression was strongly correlated with chondrogenesis in cells derived from both cartilage and bone marrow. In OA cartilage, the Angiogenesis PCR Array revealed decreased gene expression in the majority of anti-angiogenic factors, whereas the gene expression of angiogenic-related cytokines increased (Figure 1B). ACCs over-expressing ChM-1 appeared to protect chondrocytes from TNFα induced hypertrophy, and was commensurate with decreased gene and protein level of *MMP13, COL10* and *VEGF*; whereas siRNA-decreased ChM-1 levels resulted in the loss of chondrogenic marker expression and chondrocytes undergoing hypertrophy and becoming more sensitive to inflammatory cytokines. LV-ChM-1 induced over-expression of ChM-1 in the rat model resulted in delayed OA development. It was noted that ChM-1 delayed HIF-2α nuclear translocation at an early time-point and decreased the transcriptional activity of HIF-2α on *VEGF* and *MMP13*.
Conclusions: These findings demonstrate that ChM-1 is a potent anti-angiogenic factor that is essential to maintain the chondrocyte phenotype and prevent these cells from undergoing hypertrophy during OA development by inhibition of the HIF-2α mediated angiogenic pathway. Thus, this study shows that anti-angiogenic factors such as ChM-1 are essential for the maintenance of chondrocyte homeostasis, prevent hypoxia induced angiogenesis in cartilage, and protect chondrocyte from inflammatory cytokines induced hypotrophy.